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We are investigating how the neuroendocrine system affects macrophage activation and how products derived from activated macrophages affect animal behavior. We have shown that interferon-γ effectively counteracts the suppression in the synthesis of macrophage-derived TNF-α caused by both glucocorticoids and transforming growth factor β2 (Beta-2). Furthermore, the decline in synthesis of TNF-α and secretion of superoxide anion that occurs in macrophages from aged rats can be significantly reversed by syngeneic pituitary grafts. Macrophage products are also responsible for some aspects of sickness behavior, as defined by peripheral and central injections of IL-1 inducing conditioned taste aversion in both endotoxin sensitive and resistant mice. Indomethacin and aspirin do not block the effects of IL-1 on conditioned taste aversion, but they both inhibit the reductions in social exploration and schedule-controlled behavior of rats injected peripherally with IL-1. These data support the idea that macrophage products are responsible for behavioral symptoms of illness following a bacterial infection, and that macrophage activation is regulated by hormones from the pituitary gland.

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INTRODUCTION

The discovery of communication signals between the brain and the immune system represents a very significant advance in the field of medical sciences during the last decade. Thanks to the availability of recombinant technology, many of the soluble proteins which are synthesized and released by macrophages and lymphocytes during the course of an immune response have become available for investigation. It is now apparent that besides their role in the coordination of the different components of the immune response, these molecules, which are collectively designated as cytokines, are also able to act as hormones on distant target organs such as the brain. In addition, the production of cytokines at the level of the immune system is itself regulated by the neuroendocrine and autonomic nervous systems.

The overall objective of the project is to increase our knowledge about the pathophysiological significance of the reciprocal interactions that occur between the brain and the immune system. In particular, this project has two major objectives:

(a) To understand the efferent limb of the immune-central nervous system interactions by investigating how pituitary hormones and adrenal hormones affect the activities of macrophages.

(b) To understand the afferent limb of immune-central nervous system interactions by determining the mechanisms of sickness behavior induced by cytokines released by macrophages during the course of an infectious episode.

In this report, we summarize the methods that have been developed for this purpose and the results that have been obtained during the first year of the project. The relevant work has been carried out concurrently in Bordeaux, France and in Urbana, Illinois.

I. Effects of Hormones on Macrophage Activation

Macrophages serve a number of important regulatory and functional roles in the immune system. Macrophages kill facultative intracellular bacteria, phagocytose and process antigens, present these processed antigens on the surface membrane to T cells in conjunction with class II molecules of the major histocompatibility complex (MHC), kill tumor cells and serve as accessory cells for lymphocytes. The tumoricidal and bactericidal properties of macrophages can be enhanced, leading to development of activated macrophages. Macrophages release over 100 defined molecules and possess receptors for at least 30 different ligands. Macrophages are extremely active, with

endocytosis of even resident macrophages leading to turnover of the entire plasma membrane every 30 minutes.

Given the critical importance of macrophages in regulating and coordinating the immune response and playing a key role in killing bacteria, parasites and neoplastic cells, part of this project is devoted to studying how products of the immune system interact with pituitary and adrenal hormones in the regulation of macrophage activation.

Role of Transforming Growth Factor-β2 (TGF-β2), Glucocorticoids and Interferon-γ on the Secretion of TNF-α

Regulation of macrophage activation takes place both locally and at distance, by factors originating from the immune system and acting in a paracrine or in an autocrine fashion, and by hormonal products originating from the neuroendocrine system. In the first category belongs TGF-β2, a 25-kDa disulfide-linked dimer that was originally isolated and purified from human platelets. This cytokine is also synthesized and released by macrophages, B lymphocytes and T lymphocytes. It inhibits the activation of T lymphocytes and suppresses certain macrophage activities. In the second category belong glucocorticoids that are typically released during infections, under the effect of monokines such as tumor necrosis factors-α (TNF-α) and interleukin-1 (Il-1) on hypothalamic and/or pituitary targets involved in the regulation of the pituitary-adrenal axis.

To improve our understanding of the interaction between these local and distant modes of regulation, we decided to study the interaction between glucocorticoids and TGF-β2 in the down regulation of macrophages activated by the exogenous macrophage activating factor, interferon-γ (IFN-γ).

We have found that TGF-β2 is as effective as glucocorticoids in suppressing the production of TNF-α by lipopolysaccharide (LPS)-stimulated macrophages, and this inhibition can be abrogated by exogenous IFN-γ. Porcine alveolar macrophages triggered with LPS produce TNF-α, as identified by complete blocking of cytotoxicity on WEHI 164 clone 13 cells in macrophage supernatants by a monoclonal antibody to human TNF-α. Platelet-derived porcine TGF-β2, at a concentration of 4 nM, inhibited LPS-induced production of TNF-α by 93%. Dexamethasone was equally as effective as TGF-β, suppressing TNF-α production by 86% at a concentration of 4 nM. The natural but less potent glucocorticoid, cortisol, inhibited TNF-α production by 100% at a 100-fold higher concentration (400 nM). Recombinant porcine IFN-γ consistently primed LPS-triggered macrophages for increased production of TNF-α by 50-100%, and this priming was totally blocked by a polyclonal antibody to IFN-γ. Furthermore, the suppression in LPS-induced

production of TNF-α caused by TGF-β, dexamethasone and cortisol could be reversed by addition of IFN-γ. These data show that alveolar macrophages can be effectively primed by IFN-γ even in the presence of moderately suppressive doses of TGF-β and anti-inflammatory steroids. The results further suggest that administration of IFN-γ may be more beneficial to the immunocompromised host. Based upon data that will be discussed below, we are now pursuing the possibility that growth hormone may also reverse the immunosuppressive effects of TGF-β2 and glucocorticoids on macrophage activation.

The Suppression in Macrophage Priming of Aged Rats is Reversed by Syngeneic Pituitary Grafts

The typical decrease in immunity that occurs during aging can be used as a baseline to study the physiological relevance of the set of neuroendocrino-immuno interactions that are involved in regulation of macrophage functions. Old animals and humans are more susceptible to infections and tumor growth. Although it seems logical that some kind of defect in macrophage function could explain these age-associated changes in host resistance, current reports on macrophage activities during aging are inconsistent. We postulated that a defect in macrophage activation may occur in aged animals by their failure to respond to a classic priming signal, recombinant IFN-γ. We have now shown that macrophage responses to recombinant IFN-γ decline during aging, as measured by two criteria of macrophage activation: O_2^- and TNF-α secretion. The production of O_2^- by macrophages in response to opsonized-zymosan and recombinant rat IFN-γ was 75% lower in 24-mo-old rats than in young, 3-mo-old rats. Furthermore, the secretion of TNF-α in response to LPS and IFN-γ was almost absent in macrophages from aged rats. Production of both O_2^- and TNF-α by resident peritoneal macrophages from specific-pathogen-free aged rats in response to priming and triggering stimuli was partially or fully restored by implantation of syngeneic pituitary grafts from young rats. These data demonstrate that macrophages from aged rats are defective in their response to a priming signal induced by IFN-γ, and they suggest that impaired macrophage responses during aging may be reversible by hormones secreted by the pituitary gland. These findings probably at least partially explain why elderly persons cannot develop adequate fevers and are at risk for certain bacterial infections and neoplastic diseases. We are now trying to obtain adequate quantities of recombinant human growth hormone and prolactin to conduct _in vivo_ experiments to determine if either of these molecules will reverse the defect in priming signals of macrophages from aged rats.

II. Effects of Cytokines on Animal Behavior

Signals originating from the immune system during the course of an infection can act on distant target organs such as the central nervous system. Such effects play an important role in the regulation of physiologic responses to infection and inflammation. Fever is a typical example. This adaptive response to pathogens is induced by cytokines, such as IL-1, TNF-α and IL-6 that are released by macrophages and that act on the hypothalamus to increase the thermoregulatory set point. There is also evidence that IL-1 and other inflammatory cytokines are responsible for the very profound behavioral changes that are characteristic of sickness. These changes include sleepiness, reduced locomotion, decreased food and water intake, disappearance of body care activities and loss of interest in social activities. Sickness behavior is adaptive in the sense that it contributes to the reduction of thermal losses and minimizes the risk of predation for a weakened organism during the course of an infectious episode.

IL-1 and TNF-α are also able to act on the hypothalamic-pituitary system to affect the secretion of various hormones such as pituitary-adrenal hormones, growth hormone, prolactin and gonadotrophic hormones. As pointed out in the first section of this report, these hormones modulate the synthesis and release of cytokines by macrophages.

Conditioned Taste Aversion

If an animal feels sick after it has ingested a food with a new taste, it will attribute the sickness to the food it has ingested. This conditioned taste aversion (CTA) can therefore be used to assess the sickness-inducing properties of cytokines produced by macrophages. Although there is no strict relationship between the ability of a treatment to induce CTA and its sickness-inducing properties, this technique has proven to be useful to study in an objective way the perception of internal changes brought about by the treatment under investigation. In a series of experiments carried out with mice and rats, we demonstrated that LPS induces powerful conditioned aversion to the taste of a saccharin solution paired with peripheral injection of this endotoxin. CTA was accompanied by reduced fluid intake, loss of body weight and pituitary-adrenal activation in endotoxin-sensitive mice but not in endotoxin-resistant mice. In contrast, both lines of mice were sensitive to the CTA-inducing effect of Il-1 (recombinant human Il-1β). In rats we confirmed our previous results showing that Il-1 (recombinant rat Il-1) induced CTA when it was injected peripherally and we obtained similar results after injection of Il-1 into the lateral ventricle (icv) at doses which were one hundred fold less than those injected at the periphery. In both cases, the intensity of aversion to saccharin was proportional to the extent of weight loss induced by the treatment.

Schedule-Controlled Behavior

To study more thoroughly the time course of the sickness and recovery processes induced by LPS and Il-1, we selected performance on a fixed ratio-10 schedule as a behavioral endpoint. Food-deprived rats were trained to press a lever for a food pellet in a Skinner box and they were rewarded after 10 consecutive lever presses. After stabilization of response rates, rats were injected with saline or various doses of the treatment under investigation and their performance was assessed in 5-min sessions run at various intervals after administration of the treatment. While the preformance of saline-treated animals remained stable over time, the performance of Il-1 treated rats decreased in a dose- and time-dependent manner, with a maximum disruption 1-2 hrs following ip injection of Il-1 and a full recovery by 8-24 hrs.

Social Exploration

Another way to assess sickness is to study its interference with social exploration. The interest of the test animal for a conspecific is assessed by introducing a juvenile in its home cage and measuring the amount of time the test animal investigates the juvenile during a 5-min session. The use of a juvenile as a social stimulus offers the advantage of minimizing the risks of intrusion of aggression or sexual behavior in thesequence of interactive behaviors the test animal displays during the observation session. Both mice and rats show consistent and reliable investigation of the juvenile when tested repeatedly over time and this behavior was disrupted in a very sensitive way by LPS and Il-1, with the same time course as the one observed in FR-10 trained rats. Using recombinant human Il-1 beta, the doses that attenuated social investigation were 1-5 ug ip and 1-5 ng icv. Endotoxin-resistant mice failed to exhibit changes in social investigation following peripheral treatment with LPS but they fully responded to Il-1.

Role of Endogenous Pyrogens in LPS-Induced Sickness Behavior

The fact that LPS was not effective in endotoxin-resistant mice, in contrast to Il-1 suggests that LPS induces behavioral changes through the production of Il-1 beta and other cytokines by macrophages. Further evidence in favor of this possibility was provided by the demonstration that pretreatment with dexamethasone at doses known to block the synthesis and release of cytokines from macrophages (5 mg/kg, twice at 24 hrs interval) blocked the decrease in social investigation induced by LPS in endotoxin-sensitive mice.

Role of Prostaglandins in Sickness Behavior

Many of the effects of Il-1 on target organs are mediated by prostaglandins. This is particulary true for its central action

since the physico-chemical characteristics of this molecule make it unlikely to cross the blood-brain barrier. The role of prostaglandins in the behavioral effects of Il-1 was assessed by pretreating rats or mice submitted to the CTA procedure, to the FR10 schedule of food reinforcement or to the social exploration test with various inhibitors of the synthesis of prostaglandins (aspirin, indomethacin, piroxicam). Aspirin and indomethacin were unable to block the CTA induced by peripheral or central injections of Il-1 in rats and actually the doses of aspirin and indomethacin used had by themselves aversive effects. In contrast, indomethacin (10 mg/kg ip) and piroxicam (10 mg/kg ip) were both able to block the effects of peripherally injected Il-1 on social exploration in mice and on schedule-controlled behavior in rats. Further experiments are necessary to determine if the same treatments can block the effects of centrally-injected Il-1 and to delineate the site of action of the prostaglandins mediators.

Role of CRF in Sickness Behavior

Because of the profound effects of Il-1 on hypothalamic CRF and the demonstration that CRF is involved in the pyrogenic effect of this cytokine, the contribution of CRF to the behavioral action of Il-1 was investigated. Rats were injected with either 5 ug alpha-helical CRF(-9-41) to block CRF receptors, or 0.5 ug CRF. The disruptive effects of 3 ug Il-1 on schedule controlled behavior were neither augmented nor attenuated by CRF or alpha-helical CRF(-9-41). These results suggest that the behavioral effects of peripherally injected Il-1 are not mediated via its action on CRF release. Whether the same conclusion applies to centrally injected Il-1 must still be investigated.

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